IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Solomon S. Steiner, Rodney J. Woods, and Joseph W. Sulner

Serial No.: 10/719,734 Art Unit: 1616

Filed: November 21, 2003 Examiner: Alstrum Acevedo, James Henry

For: *PURIFICATION AND STABILIZATION OF PEPTIDE AND PROTEIN PHARMACEUTICAL AGENTS*

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

I, Marshall Grant, hereby declare:

1. I am a Senior Director in the Research & Development group at MannKind Corp., the assignee for the above-referenced application. I obtained a Ph.D. degree in Chemical Engineering in 1992 from Princeton University. After graduation, I worked at Exxon Corporation and returned to the Chemical Engineering Department at Princeton University as a postdoctoral fellow in 1994. From 1996 to 2001, I held the position of Assistant Professor in the Chemical Engineering Department at Yale University. Since 2001, I have been at MannKind Corporation. I have over 6 years of experience in the field of drug formulation and delivery. My Curriculum Vitae is attached.

2. As a Senior Director of the R & D group, I have designed and established the processes used for manufacturing of the Company's products relating to diketopiperazines as a drug delivery system. I presently oversee, and therefore, I am daily involved in the development and

testing of the Company's drug/diketopiperazine delivery systems, including products containing insulin. I also have extensive knowledge of the subject matter relating to the Company's present application and prior patents.

3. I have read and understand the Office Action mailed on February 7, 2007 and the references cited therein.

4. The claims in the application refer to a method for delivering monomeric or dimeric insulin to a patient in need thereof by administering to the patient a delivery formulation for the monomeric or dimeric insulin comprising an effective amount of insulin complexed with a diketopiperazine derivative. The claims specify that the delivery formulation is prepared by complexing the insulin with microparticles of the diketopiperazine derivative, wherein the diketopiperazine derivative has the formula 2,5-diketo-3,6-di(4-X-aminobutyl)piperazine, wherein X is selected from the group consisting of fumaryl, succinyl, maleyl, and glutaryl.

5. I understand the Examiner cited U.S. Patent No. 5,352,461 to Feldstein *et al.* ("the '461 patent") and U.S. Patent No. 5,503,852 to Steiner *et al.* ("the '852 patent") in his novelty rejection. Both the '461 patent and the '852 patent disclose encapsulating an active agent in diketopiperazine microparticles, using co-precipitation to capture the agent within the diketopiperazine precipitate (*see* the '461 patent, col. 5, lines 39-43 and the '852 patent, col. 9, line 55 to col. 10, lines 8). The Examiner acknowledges that neither the '461 patent nor the '852 patent disclose complexing insulin with microparticles of a diketopiperazine derivative (*see* Office Action mailed February 7, 2007, page 3). However, the Examiner asserts that complexation inherently occurs when a diketopiperizine and insulin are "closely associated with one another, as is the case in encapsulation." (*Id.*)

6. The following experiments described herewith demonstrate that complexation is not an inherent result of an encapsulation process.

7. **Materials and Methods**

Fumaryl-diketopiperizine ("FDKP") and insulin containing particles were prepared by four different methods. In three of these experiments, insulin was "encapsulated" into FDKP/insulin particles using "co-precipitation" methods described in the '461 and '852 patents. In another experiment, the insulin and preformed insulin-free FDKP particles were complexed to form FDKP/insulin-containing particles using a "complexation" procedure as disclosed in the instant application.

8. In the first method, FDKP and insulin were co-precipitated ("Co-precipitation 1"). In Co-precipitation 1, the particles were prepared in general accordance with the co-precipitation process described in the '852 patent at column 15, Example 3, except that acetic acid was used in place of citric acid, aqueous ammonia solution was used instead of sodium bicarbonate, and recombinant human insulin was used instead of porcine insulin. Recombinant human insulin was encapsulated in FDKP by first dissolving the diketopiperazine in dilute ammonia solution to form a solution containing 25 mg diketopiperazine/ml and then mixing the FDKP solution with an equal volume of acetic acid solution containing recombinant human insulin at a concentration of 2.5 mg insulin/ml to form a white precipitate. The product was collected by centrifugation, washed three times with deionized water, flash-frozen in liquid nitrogen and then dried by lyophilization.

9. In the second method, FDKP and insulin were co-precipitated ("Co-precipitation 2"). In Co-precipitation 2, the particles were prepared in accordance with the general process described in the '852 patent at column 9, lines 58-63, except in Co-precipitation 2 acetic acid was used in

place of citric acid. Recombinant human insulin was encapsulated in FDKP by dissolving both FDKP and insulin in dilute ammonia to produce an FDKP/insulin solution containing 25 mg/ml FDKP and 2.5 mg/ml insulin. The FDKP/insulin solution was then mixed with an equal volume of acetic acid solution to form microparticles. The product was collected by centrifugation, washed three times with deionized water, flash-frozen in liquid nitrogen and then dried by lyophilization.

10. One of the experiments used a variation of the above method, ("Co-precipitation 2a"). In Co-precipitation 2a, the particles were prepared in accordance with the general process described in the '852 patent at column 9, lines 58-63, except in Co-precipitation 2a, acetic acid was used in place of citric acid. Recombinant human insulin was encapsulated in FDKP by first dissolving FDKP in dilute ammonia, and combining the FDKP solution with a solution of insulin dissolved in phosphoric acid to yield an FDKP/insulin solution containing 25 mg/ml FDKP and 5 mg/ml insulin. The FDKP/insulin solution was then mixed with an equal volume of acetic acid solution to form microparticles. The product was collected by centrifugation, washed three times with deionized water, flash-frozen in liquid nitrogen and then dried by lyophilization.

11. In the final method, insulin was adsorbed onto a preformed FDKP particle ("Complexation"). In Complexation, the particles were prepared in accordance with the method described in the present application, U.S.S.N. 10/719,734, on page 16, lines 3-7. Insulin-free microparticles of FDKP were prepared by mixing a 25 mg/ml FDKP solution with an equal volume of acetic acid solution to form microparticles. The particles were collected by centrifugation, washed with deionized water, and dried by lyophilization to obtain a bulk powder. The FDKP microparticles were then suspended at 50 mg/ml in deionized water. An insulin stock solution was prepared and transferred to the suspension to yield a final insulin

concentration of 5 mg/ml, pH 3. The suspension was flash-frozen in liquid nitrogen and dried by lyophilization to yield diketopiperazine microparticles complexed with insulin.

12. In one set of experiments, insulin recovery, insulin stability and variability of insulin content were determined for dry powders prepared according to "Co-precipitation 1," "Co-precipitation 2," and "Complexation." The results of these experiments are summarized in Table 1. In an independent set of experiments, powders prepared by "Co-precipitation 2a" and "Complexation" were used in insulin stability studies to compare the two types of particles. The results from this experiment are summarized in Figure 1.

13. **Results**

Insulin recovery and insulin stability were determined for each of the three sets of microparticles by reverse-phase HPLC assay of the dissolved powders. Variability in insulin content was determined using additional samples of batches that had been manufactured for use in clinical trials.

TABLE 1

Manufacturing Method	Insulin recovery (% target insulin assay value)	Insulin stability (%insulin loss after 10 days at 40°C/75%RH)	Insulin content variability
Co-precipitation 1	87.9	27±1 (N=3 replicates)	<±20% of target *
Co-precipitation 2	53.8	34±2 (N=3 replicates)	Not tested
Complexation	>99	15±4 (N=5 replicates)	<±10% of target

* Insulin content variability was measured from supplementary samples from different batches of material prepared for clinical trials using this method.

14. As shown in Table 1, Complexation resulted in a very high level of insulin recovery (greater than 99%) and produced particles with the most stable insulin (15% loss). In contrast,

Co-precipitation 2 resulted in poor insulin recovery (54%), with the least stable insulin (34% loss). Co-precipitation 1 resulted in a high level of insulin recovery (87.9%), with less stable insulin (27%) than that obtained using Complexation. Although the particles produced using Co-precipitation 1 were more stable than those produced using Co-precipitation 2, they were significantly less stable than the particles produced by Complexation. The insulin stability test demonstrates that the insulin in particles produced by Co-precipitation 1 degraded about twice as fast as the insulin complexed to pre-formed FDKP microparticles.

15. Insulin content variability was also tested. In this test, Complexation always produced particles with insulin content within 10% of the target amount. In contrast, Co-precipitation 1 had greater variability in insulin content and produced particles with insulin content within 20% of the target amount. The variability of Co-precipitation 2 was not investigated because the insulin recovery was so poor.

16. The substantial difference in insulin stability in microparticles produced by Complexation versus Co-precipitation was confirmed in a separate experiment in which powder samples prepared by Co-precipitation 2a were compared with powder samples prepared by Complexation. As seen in Figure 1, the data show that after 10 weeks of incubation at 40° C and 75% relative humidity, the insulin present in particles produced by Co-precipitation 2a underwent about 4 times as much degradation (60% loss), than the insulin present in particles produced by Complexation (15% loss). Results from duplicate samples of powders (Figure 1) show that samples produced by complexation are more stable than those produced by co-precipitation and that there was no appreciable difference between samples stored under nitrogen (open plot symbols), or in an open container (filled plot symbols). These results provide independent confirmation of the results reported in Table 1.

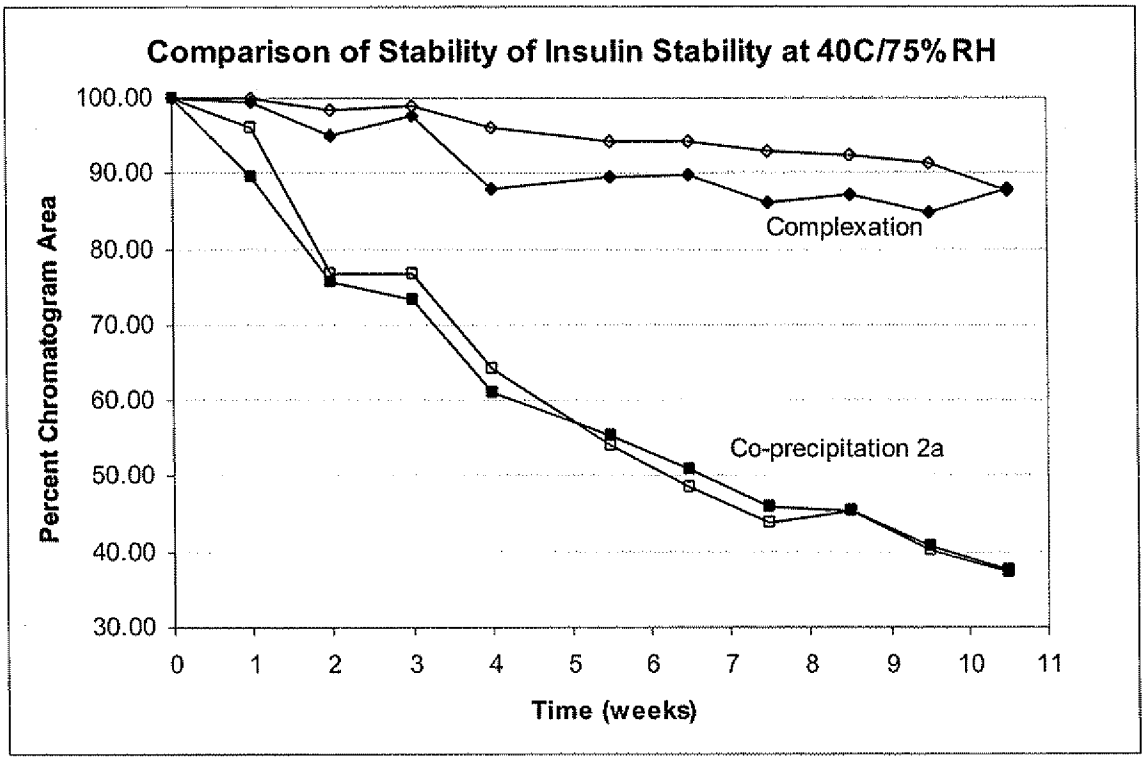

Figure 1

17. These results demonstrate that the co-precipitation-based encapsulation methods described in the '461 and '852 patents can produce microparticles with different properties than those produced using a complexation method. As shown by the experiments described herein, the insulin in the microparticles produced by the complexation method was more stable than in microparticles produced using the cited prior art methods. Thus the disclosure of the co-precipitation-based encapsulation methods of the '461 and '852 patents does not inherently disclose complexation.

18. I declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true, and further, that the statements are made with the knowledge that willful false statements are punishable by fine or

imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 09/19/07

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Education

Ph. D. in Chemical Engineering, Princeton University, Princeton NJ, January 1992.
M. A. in Chemical Engineering, Princeton University, Princeton NJ, January 1986.
B. S. in Chemical Engineering with highest distinction, Purdue University,
West Lafayette IN, May 1984.

Experience

MannKind Corporation, Danbury CT — 7/2001 – present

Director, Formulations Research (2001-2006)
Senior Director, Formulation Development (2006-present)

- Process and product development of dry powder formulation of insulin for pulmonary delivery
  - Increased production capacity by more than 400% to meet clinical demand.
  - Improved aerodynamic performance.
  - Extended room temperature shelf life of product.
  - Contributed to CMC documents for submission to FDA and other regulatory agencies.
  - Assisted in pharmacokinetic analysis to support understanding of clinical data.
  - Participated in pre-clinical studies to evaluate formulated drug products.
- Directed feasibility studies for prospective joint ventures.
- Provided technical assessments to upper management as needed.

Yale University, New Haven CT — 1996 – 2001

Assistant Professor, Department of Chemical Engineering

- Crystallization of proteins
- Thermodynamics of protein interactions in solution and protein-surface interactions
- Colloidal phenomena

Boehringer Ingelheim Pharmaceuticals, Ridgefield CT — 1/2000 – 5/2000

Visiting Fellow, Chemical Development Department

- Solvent screening for pharmaceutical crystallization
- Drug solubility

Villanova University, Villanova, PA — 8/1995 - 12/1995

Assistant Professor (visiting), Department of Chemical Engineering

Princeton University, Princeton, NJ 1994 - 1995
Post-Doctoral Research Associate, Department of Chemical Engineering

- Experimental study of particle migration in flowing viscoelastic fluids
- Experimental study of polymer-induced flocculation of particles

Exxon Production Research Company, Houston, TX 1991 - 1994
Research Engineer (11/91 - 12/92); Senior Research Engineer (12/92 - 1/94)

- Developed and analyzed probabilistic models of oil and gas reservoirs to estimate uncertainty in oil-in-place and recovery efficiency

Publications

M.L. Grant, "Nonuniform charge effects in protein-protein interactions." *Journal of Physical Chemistry B* **105** (2001) 2858-2863.

M.L. Grant, "Effects of thermodynamic nonideality in protein crystal growth." *Journal of Crystal Growth* **209** (2000) 130-137.

M.L. Grant and D.A. Saville, "Long-term studies on tetragonal lysozyme crystals grown in quiescent and forced convection environments." *Journal of Crystal Growth* **153** (1995) 42-54.

M.L. Grant and D.A. Saville, "Electrostatic interactions between a nonuniformly charged sphere and a charged surface." *Journal of Colloid and Interface Science* **171** (1995) 35-45.

M.L. Grant and D.A. Saville, "Colloidal interactions in protein crystal growth." *Journal of Physical Chemistry* **98** (1994) 10358-10367.

M.L. Grant and D.A. Saville, "The role of transport phenomena in protein crystal growth." *Journal of Crystal Growth* **108** (1991) 8-18.

S.M. Rekhson, M.L. Grant and H.F. Peckman, "Three modes of glass failure in the glass transition region." *Glastechnische Berichte,* **56K** (1983).

Presentations, Posters and Published Abstracts

M. Grant, E. Harris, A. Leone-Bay, and K. Rousseau, "Technosphere®/Insulin: Method of Action." Diabetes Technology, Atlanta GA 2006.

A.H. Boss, M.L. Grant, and W.W. Cheatham, and A.H. Boss "Both insulin sensitivity and maximal glucose elimination rate are reduced in type 2 diabetes." American Diabetes Association, San Diego CA, 2005.

M.L. Grant and W.W. Cheatham, "Mimicry of the early phase insulin response in humans with rapidly available inhaled insulin accelerates postprandial glucose elimination compared to slower bioavailable insulin." American Diabetes Association, San Diego CA, 2005.

K.A. Leiner, P. Krueger, K. Daukas, P. Menkin, I. Trantcheva, M. Jackson, T. Vaccaro, K. Rousseau, I. Carballo, O. Gelber, M. Grant, and C. Gelber, "The pharmacokinetic profile of Technosphere®/Insulin administered by inhalation in the rat." American Diabetes Association, Orlando FL, 2004.

M. Grant, P. Menkin, I. Trantcheva, K.A. Leiner, and C. Gelber, "Distribution of ^{14}C-labeled Technosphere® particles following intra-tracheal, liquid instillation in the Sprague-Dawley Rat." American Diabetes Association, Orlando FL, May 2004.

K. Rousseau, I. Carballo, P. Robustelli, M. Grant, and C. Gelber, "Drug delivery by fumaryl diketopiperazine particles: Evidence for passive transport." American Diabetes Association, Orlando FL, May 2004.

M.L. Grant, "Implications of thermodynamic nonideality in protein crystallization." American Institute of Chemical Engineers National Meeting, Dallas TX, Nov. 1999.

M.L. Grant, "Colloidal interactions with heterogeneously charged particles." American Institute of Chemical Engineers National Meeting, Miami FL, Nov. 1998.

M.L. Grant, "Thermodynamics of dilute protein solutions from colloidal interactions." American Institute of Chemical Engineers National Meeting, Los Angeles CA, Nov. 1997.

M.L. Grant, "Protein crystal growth: Molecular interactions and transport phenomena." 1997 Yale-Hewlett Packard Conversazione on Advances in Analytical Biotechnology and Related Areas, New Haven CT, May 1997.

M.L. Grant, "Modeling protein-protein interactions in solution: An application to crystallization." American Institute of Chemical Engineers National Meeting, Chicago IL, Nov. 1996.

C.V. Deutsch and M.L. Grant, "Analysis of random function models in terms of spatial entropy." Society of Industrial and Applied Mathematics (SIAM) Minisymposium on Reservoir Characterization, Houston TX, 1993.

M.L. Grant, "The hanging drop revisited: Protein concentration gradients in an unstirred drop." 3rd Joe Wheeler Workshop on Protein Crystal Growth, Rogersville AL, 1988.

M.L. Grant and D.A. Saville, "Puzzling aspects of protein crystal growth." 2nd Joe Wheeler Workshop on Protein Crystal Growth, Rogersville AL, 1987.

S.M. Rekhson and M.L. Grant, "Fracture of glass under tension due to void formation and growth." 86th Annual Meeting of American Ceramic Society, Pittsburgh PA, 1984.

S.M. Rekhson and M.L. Grant, "Fracture of liquid glass." 85th Annual Meeting of the American Ceramic Society, Chicago IL, 1983.

Professional Activities

- Member, American Institute of Chemical Engineers (AIChE) and American Association of Pharmaceutical Scientists (AAPS)
- Chaired/Co-Chaired sessions on the "Crystallization of Biological and Pharmaceutical Molecules" at the AIChE Annual Meetings in 1998, 1999, and 2000.
- Chaired/Co-Chaired sessions on "Colloidal Dispersions" at the AIChE Annual Meetings in 1999 and 2000.
- Referee: *Journal of Crystal Growth, Journal of Colloid and Interface Science.*